

342

RAPAMYCIN PREVENTS GVHD IN MOUSE MODEL THROUGH NOVEL MECHANISM: INCREASE IN REGULATORY T CELLS

Palmer, J.M., Chen, B.J., DeOliviera, D., Le, N., Chao, N.J. Duke University, Durham, NC.

Rapamycin (RAPA) is an immunosuppressant that has been used for many years for prevention of rejection in solid organ transplantation. It also appears to have some impact on acute graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). In previous experiments done in our lab, RAPA significantly improves survival in GVHD mouse model. Recently, it has been found that RAPA can increase numbers or enhance survival of regulatory T cells. In this experiment we further investigate the mechanisms behind RAPA in a murine GVHD model. In several experiments, B10. D2 bone marrow and splenocytes were injected into Balb/c mice. The mice had samples of peripheral blood and ear biopsies evaluated at two, four and six weeks post-transplantation. Regulatory T cells were analyzed based on intracellular FoxP3 expression via FACS or immunohistochemistry. At all three time points in the peripheral blood, the percent of regulatory T cells in total CD4+ cells was not significantly different. On days 14 and 28 there were significant differences seen in the ear biopsies, both in morphologic appearance and percent FoxP3 positive cells of mononuclear cells. On day 14 there was more inflammation in the control arm compared to the RAPA treated mice, however, there was a cellular infiltrate present in both. The percentage of FoxP3 positive cells was 13% in the treated arm as compared to 2.5% in the control arm ($p = 0.02$). On day 28, there was a decrease in cellularity in the control arm as compared to the RAPA arm, however in the RAPA arm there was a significant increase in percent of FoxP3 positive cells as compared to control arm: 9% vs 1.2% ($p = 0.04$). These results suggest that the mechanism through which RAPA prevents GVHD in this model is not only immunosuppression but also through the effects of regulatory T cells. It appears that the effect is mediated locally in the tissues, rather than through circulating regulatory T cells.

343

INITIAL THERAPY FOR ACUTE GRAFT-VERSUS-HOST DISEASE WITH "LOW-DOSE" PREDNISONE (1 mg/kg/day) INSTEAD OF "STANDARD-DOSE" PREDNISONE (2 mg/kg/day) DOES NOT COMPROMISE MAJOR OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

Mielcarek, M., Storer, B., Martin, P.J. Fred Hutchinson Cancer Research Center, Seattle, WA.

The optimal glucocorticoid dose for initial treatment of acute graft-versus-host disease (GVHD) remains to be defined. We hypothesized that initial treatment with "low-dose" glucocorticoids (equivalent to prednisone, 1 mg/kg/day) would not compromise major transplantation outcomes compared to treatment with "standard-dose" glucocorticoids (equivalent to prednisone, 2 mg/kg/day). In a retrospective review, we identified 347 patients with acute GVHD who were treated initially with low-dose glucocorticoids and 386 who were treated at standard doses. All patients were at least 18 years of age and had transplants between 2000 and 2005 at a single institution. Fifty-eight (17%) of the 347 patients in the low-dose group subsequently received standard-dose treatment after inadequate response to low-dose treatment. The mean cumulative prednisone-equivalent doses across time in the low-dose group were reduced by approximately 48% compared to the standard-dose group. After adjusting for donor type (unrelated *vs* other), HLA-mismatch (any *vs* other), patient age (continuous), conditioning intensity (myeloablative *vs* nonmyeloablative), donor/recipient gender disparity, prophylactic use of ursodeoxycholic acid and tacrolimus, GVHD grade at onset of therapy and use of beclomethasone dipropionate (time-dependent covariate) in multivariate analysis, we found no statistically significant differences in outcomes in the low-dose group compared to the standard-dose group: overall mortality (hazard ratio [HR], 0.99; 95% confidence interval [CI], 0.8–1.3), relapse (HR, 1.14; 95% CI, 0.8–1.6), non-relapse mortality (HR, 0.92; 95% CI, 0.7–1.3). Although the proportion of patients receiving secondary therapy (including an increase

from low dose to standard dose) was similar in the two groups, the onset of secondary therapy occurred sooner in the low-dose group (HR, 1.94; 95% CI, 1.3–2.8). Endpoints of prednisone-related morbidity were not assessed in this study. Results were similar in subgroups of patients with GVHD characterized by rash involving $\leq 50\%$ body surface area and stool volumes ≤ 1.0 L with or without anorexia, nausea and vomiting ($n = 425$) and in those with more severe GVHD ($n = 308$). We conclude that, despite a nearly 50% reduction in the cumulative glucocorticoid dose across time, initial glucocorticoid treatment at a prednisone-equivalent dose of 1 mg/kg/day was not associated with inferior outcomes when compared to initial treatment with standard-dose glucocorticoids.

344

OFF-THE-SHELF TUMOR IMMUNOTHERAPY WITH GENETICALLY ENHANCED ALLOGENEIC T-CELL PRECURSORS

Zakrzewski, J.L.¹, Sub, D.¹, Markley, J.C.², Smith, O.M.¹, King, C.¹, Goldberg, G.¹, Jenq, R.¹, Holland, A.M.¹, Grubin, J.¹, Cabrera-Perez, J.¹, Lu, S.X.¹, Rizzuto, G.¹, Sant'Angelo, D.B.¹, Riviere, I.², Sadelain, M.², Zuniga-Pfucker, J.C.³, van den Brink, M.R.M.¹. ¹Memorial Sloan Kettering Cancer Center, New York, NY; ²Memorial Sloan Kettering Cancer Center, New York, NY; ³University of Toronto, Toronto, ON, Canada.

T cell deficiencies can occur in many physiological and pathophysiological settings such as aging, malignant diseases, and cytostatic therapy. We recently reported that co-transplantation of in vitro generated T cell precursors significantly enhances T cell reconstitution after allogeneic HSCT resulting in increased graft-versus-tumor activity without graft-versus-host disease. The aim of our present study was to evaluate if allogeneic T cell precursors can safely be transferred across MHC barriers in the absence of allogeneic HSCs to improve anti-tumor activity in immunosuppressed recipients. We found that adoptively transferred allogeneic (C57BL/6) precursor cells in irradiated hosts (BALB/c) develop into fully functional allogeneic T cells characterized by a host-MHC restricted and host-tolerant T cell receptor. We show that adoptively transferred allogeneic T cell precursors significantly improve survival of BALB/c mice after irradiation (675 cGy) and enhance anti-tumor activity against A20 lymphoma and renal cell carcinoma in syngeneic HSCT recipients. Furthermore, we demonstrate the feasibility of genetic engineering of antigen-specific T cell precursors, by transducing them to express a chimeric antigen receptor (CAR) targeting hCD19. Immunotherapy with CAR-expressing T cell precursors resulted in the in vivo generation of high numbers of appropriately selected T cells expressing the CAR, which persisted for at least two months after transfer and mediated significantly enhanced anti-tumor activity (compared with CAR-negative T cell precursors) against a CAR-sensitive tumor, without any undesirable auto/alloreactivity. We conclude that T cell precursors from any donor can be used universally for adoptive immunotherapy in any immunosuppressed individual irrespective of MHC disparities. The use of allogeneic precursors cells instead of autologous cells eliminates the risk of contamination with residual malignant patient cells and allows the generation and storage of virtually unlimited quantities of precursor cells for 'off-the-shelf' immunotherapy. This procedure is not only labor and cost-effective, but it facilitates the application of gene transfer technology, to generate antigen-specific or otherwise enhanced designer cells. Adoptive transfer of allogeneic and genetically enhanced T cell precursors therefore represents a promising novel strategy for targeted 'off-the-shelf' immunotherapy in immunosuppressed patients.

345

DECREASED RISK OF ACUTE GVHD FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH THE 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE 677TT GENOTYPE

Sugimoto, K.¹, Murata, M.¹, Onizuka, M.², Inamoto, Y.², Terakura, S.¹, Kuwatsuka, Y.², Oba, T.², Miyamura, K.², Kodera, Y.², Naoe, T.¹. ¹Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; ²Japanese Red Cross Nagoya First Hospital, Nagoya, Aichi, Japan.

5,10-methylenetetrahydrofolate reductase (MTHFR) is a central regulatory enzyme in folate metabolism. A common functional polymorphism of the MTHFR gene occurs at C677T. The 677TT genotype produces an enzyme with only 30% of the activity of the wild-type (677CC) enzyme. The MTHFR polymorphism has been shown to affect the sensitivity of patients to folate-based drugs such as methotrexate (MTX) that is used for GVHD prophylaxis following allogeneic hematopoietic stem cell transplantation (HSCT). To assess the significance of C677T genotypes in HSCT using MTX as a GVHD prophylaxis, we analyzed DNA from 159 patients with a hematological disease and their HLA-identical sibling donors using PCR-RFLP method. The frequencies of CC, CT and TT genotypes in the patients were 35%, 52% and 13%, respectively, and in the donors were 30%, 62% and 8%, respectively. There was no significant difference in the distribution patterns of the C677T genotypes between patients and donors ($P = 0.19$). Multivariate analysis revealed a significantly lower incidence of grade I-IV acute GVHD in patients with the 677TT genotype (relative risk, 0.35; 95% confidence interval, 0.13–0.95; $P = 0.040$) and a non-malignant disease (0.22; 0.05–0.89; 0.034). There was a significant association between a lower incidence of grade II-IV acute GVHD and the use of bone marrow for transplantation (0.32; 0.11–0.91; 0.032). There was no association between the incidence of acute GVHD and the donor C677T genotypes. We analyzed the incidence of acute GVHD in relation to the MTHFR genotype using the Kaplan-Meier method. The incidence of grade I-IV acute GVHD in the patients with 677TT genotype was significantly lower than in those with 677CC/CT genotype (19% versus 45%, $P = 0.035$). There was a trend for a lower incidence of grade II-IV acute GVHD in patients with 677TT genotype compared with 677CC/CT genotype (5% versus 24%, $P = 0.077$). The C677T genotypes in the patient or donor were not associated with the treatment-related mortality, relapse rate, relapse-free survival and overall survival. These results suggest that greater immunosuppression by MTX due to low MTHFR enzyme activity decreases the risk of acute GVHD in recipients of allogeneic HSCT. Further studies are needed to confirm that the MTHFR C677T polymorphism can predict the outcome of HSCT using prophylactic MTX to prevent GVHD.

346

HOMEOSTATIC AND INFLAMMATORY PROCESSES CONTRIBUTE TO EL-EVATED PLASMA LEVELS OF B CELL ACTIVATING FACTOR (BAFF) IN CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD)

Hakim, F.T., Rehman, N., Dickinson, J., Beal, M., Baskar, S., Rader, C., Cowen, E., Pavletic, S., Gress, R.E. National Cancer Institute, NIH, Bethesda, MD.

BAFF, a non-redundant cytokine produced by myeloid cells, plays a critical role in the normal homeostatic maintenance, activation and function of B cells. Elevated circulating levels of BAFF, however, have been observed in systemic autoimmune disorders and, in murine models, have been linked to a failure to delete auto-reactive B cells. We similarly observed elevated plasma BAFF levels in 77 patients in an ongoing NCI CGVHD natural history protocol, with a median of 2845 pg/ml (range 92 to 17058), as compared to 556 pg/ml (range 75 to 1834) in 18 normal donors. Furthermore, in a subset of 22 patients in which severity of CGVHD could be assessed by the presence of marked erythema or sclerosis on 10 to 90% of their body surface areas (BSA), the BAFF level correlated with the percentage of affected BSA (Spearman $r = +.63$). We then explored the factors that might contribute to elevated BAFF levels. In recipients recovering from either autologous or allogeneic transplant (without GVHD) we observed the highest BAFF levels at day 0 (median of 10534 and 12240 pg/ml respectively), when B cells were severely depleted. As B cell populations recovered to normal levels post transplant, plasma BAFF concentrations declined (Spearman $r = -.80$ and $r = -.60$, respectively), consistent with homeostatic cytokine-consumption dynamics. Despite comparably high levels of BAFF (median of 11342 pg/ml) at transplant day 0 in 16 patients who later developed CGVHD, BAFF levels in the cross-sectional, natural history patient population were only moderately correlated with the degree of post transplant B cell recovery ($r = -.48$). Since inflammatory triggers can

induce elevated BAFF production by dendritic cells, we assessed plasma levels of cytokines indicative of an inflammatory process. In 34 patients, the plasma levels of IP-10 and sTNFRII correlated positively with BAFF levels ($r = +.627$ and $r = +.642$, respectively), consistent with active inflammatory processes in those CGVHD patients with elevated BAFF levels. In a multi-step regression model, the levels of circulating B cells, plasma IP-10 and sTNFRII combined to strongly predict BAFF levels ($R = .834$). These findings suggest that both homeostatic recovery of B cell populations consuming BAFF and inflammatory cytokine cascades initiated by donor-anti-host reactivity combine to regulate BAFF levels post transplant.

347

CORRELATION BETWEEN FOXP3 GENE POLYMORPHISMS IN DONORS, AND THE SEVERITY OF ACUTE GRAFT-VERSUS-HOST DISEASE IN PATIENTS AFTER RELATED ALLOGENEIC STEM CELL TRANSPLANTATION

Perfecto-Avalos, Y.¹, Borbolla Escobedo, J.R.¹, Villala-Martinez, L.M.¹, Scott, S.P.¹, Vela-Ojeda, J.², Gonzalez-Ramella, O.³, Baltazar-Arellano, S.⁴, Lopez-Hernandez, M.A.⁵. ¹ITESM Medical School, Monterrey, Nuevo Leon, Mexico; ²Centro Medico La Raza, IMSS, Mexico City, DF, Mexico; ³Hospital Civil de Guadalajara "Juan I Menchaca", Guadalajara, Jalisco, Mexico; ⁴HUMAE 25, IMSS, Monterrey, Nuevo Leon, Mexico; ⁵Centro Medico 20 de Noviembre, Mexico City, DF, Mexico.

Introduction: Acute Graft-versus-host disease (aGVHD) is a major complication of allogeneic stem cell transplantation (alloSCT). Risk factors include patient age, sex matching, CMV status and degree of match. Regulatory T cells are critical for immune tolerance processes such as aGVHD, and express the transcription factor FOXP3, a member of the forkhead/winged-helix family, identified as a key regulatory gene required for the development and activity of these cells. It has been suggested that genetic expression of FOXP3 is inversely correlated with the severity of the GVHD. We studied donors DNA looking for 5 polymorphisms on the promoter region of the FOXP3 gene, and we tried to correlate them with presence and degree of aGVHD. **Patients and Methods:** We studied donors of stem cells for allogeneic stem cell transplants. We looked for the presence of the following polymorphisms by PCR: POL01-5906 T/A rs2869211; POL03-3279 A/C rs3761548; POL04-2383 C/T rs3761549; POL05-1383 C/T rs2232364; POL06-924 A/G rs2232365. **Results:** Our sample consisted of 31 donors, all siblings. In them we found only 2 of the 5 FOXP3 polymorphisms, either as homozygous or heterozygous. These polymorphisms were found in 15/31 donors, with 12 being homozygous (38.7%), and 3 heterozygous (9.7%). These genes polymorphisms were POL03 y POL06. The most observed polymorphism was POL-06 with 9 cases, while POL-03 was found in 6 donors. Only sex difference and CMV status had an elevated hazard ratio for developing GVHD (HR = 1.18, CI95%: 0.18 to 7.64; $p = 0.85$) and (HR = 3.0, CI95%: 0.07 to 126; $p = 0.46$) respectively. We found no statistically significant difference in the incidence of GVHD between patients who had received cells from donor with or without a FOXP3 polymorphism ($p = 0.87$). When we analyzed the risk of presenting GVHD, results suggest that having one of the 2 positive polymorphisms of the FOXP3 gene could have a protective effect for the patient. For POL03 HR = 0.87, CI95%: 0.18 to 4.14; $p = 0.86$, and for POL06, HR = 1, CI95%: 0.37 to 2.64, $p = 0.67$. **Conclusions:** Even though our sample is still small to make conclusive remarks, we believe that our results point toward some level of protection from acute GVHD in patients receiving cells from donors expressing POL03 and POL06. It is also worthwhile mentioning the relatively high frequency of these polymorphisms, as well as the absence of the other 3.

348

HUMAN NEUTROPHIL ELASTASE STIMULATING CD4+ AND CD8+ T CELLS IS A POTENTIAL PROTEIN VACCINE FOR LEUKEMIA PATIENTS WITH DIVERSE HLA TYPES

Le, Q., Melenhorst, J.J., Hensel, N., Eniafe, R., Barrett, A.J. National Heart, Lung and Blood Institute, NIH, Bethesda, MD.